In Dialogue with Dr. Corey Nislow

iGEM CONCORDIA

ASTROYEAST 2020

Dr. Corey Nislow

Part 1: July 31st with iGEM Concordia

SUMMARY KEYWORDS

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Dr. Nislow 0:07
I'm Corey. I've been at UBC since 2012. And my title is as professor of translational genomics, but really I just work on yeast as a model system and have for about the past 25 years, starting at Stanford, then moving to University of Toronto in 2006, and then moving out to UBC, in 2012. Everything that my lab and my significant other's lab do, revolves around yeast in one way or another. And we actually have a small company that uses the technology to look at drug gene interactions as well. We've been sort of yeast people for quite a long time. It seems that it could be very fun to talk to like-minded students and trainees. And I'm really eager to hear what you guys are planning to do and how what we're doing could synergize.

iGEM Concordia, Hajar 3:38
Before we go further, Dr. Nislow. I just wanted to explain a little bit of the process for our chat today. We're doing human practices as part of the project. What we do is to consult with different stakeholders of whom researchers are part and we sort of talked to them about a project. We take feedback and we integrated it back into our project. We document this process for iGEM. Because we are in a competition and we have to be transparent.

I wanted to maybe ask you a few questions and then we're going to present our project in our database, if you're okay with that. Great. How did you become a microgravity researcher?

Dr. Nislow 7:08
I was approached by a professor at Duke University who had been working with NASA since I think shuttle flight 35. So way back in the late 1990s and it was a fortuitous event in that ever since the second shuttle disaster, there was a second backup shuttle that had to be available in case of a situation in 2003 was repeated and the shuttle that was used to bring the crew to space was not suitable for re-entry. So the idea being that there was a shuttle ready at Cape Canaveral to fly up and do a crew transfer and bring them home. And you can imagine that that brings up an issue at the end of the program. Because if you always have a second shuttle, then you're left with a perfectly good shuttle at the end of the program that has never flown.

Dr. Nislow
And so NASA went to their astronaut corps and said, 'we know there's a reason for having a backup shuttle, but we also think it would be a shame not to use it.' And so, they asked for volunteer astronauts on a much smaller crew than normal, to bring up as much science to the ISS as possible, with the full understanding that there was no backup. After the decision was made to pull the trigger on that project, NASA put out a call for projects that were ready to fly within three months. And that precludes a lot of different types of biology projects. But it was just enough time for us to miniaturize our chemo genomic screens and get them ready for flight.

Ever since that- with NASA you have to prove yourself to them to be a good partner- and that established our credibility with them. We launched on several SpaceX flights to the ISS. The situation in 2011 was mirrored when the calls came out for Artemis payloads, because again, it's a very short turnaround time in terms of getting your science ready. It had to be cheap. The environmental control and all the things that you're used to in a space payload, were not going to be available on Artemis. We kind of excel at very simple, but ultimately complex experiments. Simple to fly, complex to deconvolute. And so that's a long answer to how I first got involved in microgravity.

iGEM Concordia, Hajar 10:29
What are the most prominent changes that you observe in yeast when they're in microgravity, like what do you think are the main factors which cause these changes in regulation?

Dr. Nislow 10:41
I put them in big buckets. We see cell wall, cell membrane stresses. We see DNA repair stresses, which it's frankly hard to disentangle, deconvolute whether that's due to microgravity or the little bit of extra radiation you get in Low Earth Orbit. And then we see a big change in genes involved in redox signaling. And why? We don't know yet. The hypothesis is that when you go from the situation where you are used to having your metabolites move around and you can get away from them because you're in a situation where you know which way is up. When you're put in microgravity, those constraints are gone. The normal controls over how you get rid of oxidized proteins, lipids and things like that are changed. Those would be the three buckets I would put our, our genes in there.

iGEM Concordia 12:05
Excellent. From your experience, is there a gene or multiple genes that are considered the hallmark of microgravity induced stress response? Is there a gene, for example, that is upward or downregulated exclusively in microgravity and not in many other stressors?

Dr. Nislow 12:20
It's not as black and white as that. But there are genes, like some of the superoxide dismutases the SOD genes. It's almost like you're thinking of a drug and the effects on tumor cells versus normal cells, what's the therapeutic window? I kind of think of what's the microgravity window, you might see SOD changes in other stresses, but you see it much more in microgravity stress.

I think part is that evolution was never at play in optimizing any of these genes or genetic responses to microgravity. We're looking for a gene that's moonlighting to respond to microgravity, if that makes sense. I wouldn't put all of my bets on one particular reporter. I'd have several if your project allows for that.

iGEM Concordia, Hajar 13:35
I wanted to show you our database. I'll send you a link in the chat. We're still developing it, it's a working example of what we have so far. Okay. And I just wanted you to take a look and then tell us, as if you had been looking at an organism, would you find the information that you need in there? Are we missing some things or are there elements we could exclude?

Dr. Nislow 14:13
Great. You have gene expression changes. What are the conditions you're comparing?

iGEM Concordia, Hajar 14:32
Usually depends on the study, but we're always comparing microgravity conditions. So the experimental versus the control. And usually it tells you which type of study it is. If you go a lot more in and hit the more info button. There's a lot of good information there.

Dr. Nislow
Awesome. I just put in my favorite gene, what are the other open reading frames?

iGEM Concordia
What's your favorite?

Dr. Nislow
Well, DFR1 because it was the first gene that validated our drug screening program. But I haven't met a gene I don't like in yeast. One question I had, when you mentioned databases, will you be linking to the NASA GeneLab database?Basically all omics data

is put in there. It's typically a governmental site. It's not as user friendly as yours is, but It might be a very nice way for you to get more eyeballs on your data and your database.

Genelab.nasa.gov

iGEM Concordia,Hajar 16:50
Is there something specific that when you're looking at GeneLab that interests you when you're looking for specific?.

Dr. Nislow 17:04
Well, I think for you, in the context of your project.I would guess that it might be nice to know how orthologs of genes of interest in yeast have been studied in microgravity, either SpaceX or the shuttle or on the ISS. If you want anything beyond yeast, that would surely be a good source of data. Again, I don't really know how well curated it is, so it'll take some digging.

iGEM Concordia, Hajar 17:44
Our database also has other organisms. We started initially with yeast, but we figured that we would make it more accessible to the whole community. I think we have bacteria now, we have human studies, so we're definitely expanding and it will be open to other researchers who want to submit their data in the future. We've read your study on the survivability of yeast in spaceflight and we were interested in knowing how to incorporate it into our database, because the way we've categorized things is we have studies that looked at upregulation versus downregulation. But yours was a bit different, where you studied survivability. We are brainstorming, because we do think it's very important information to include but are trying to figure out how to include it in the database.

Well, I think I think it's fairly straightforward in that there are a lot of analogies between transcriptional profiling and fitness profiling, which is what we do. And just to remind you, basically we take the deletion collection in one form or another, that all the non essentials or all of the essentials, or a combination of the two, and they're all barcoded. So you start with all strains in equal abundance.

Then you put them through an environmental perturbation exam, for example, microgravity. And then you compare the abundance of each strain in the starting population and the ending population by counting the number of barcodes in your sample. And the number of barcodes in the sample which you count by NGS (Next-Generation Sequencing) equal is equivalent to or is a proxy for survivability. The value you get is a fold change relative to control conditions. If you wanted to keep it simple, you could report

experiment of HARVS versus clinostats versus static versus real microgravity, part of that is cost. The rotating well vessels that are available right now are prohibitively expensive. I think they are because you can't do enough time points and replicates to get robust data.

We're actually working, this is not an advertisement, but we are working on very small, disposable rotating wall vessels. That will be beta tested in the lab. It got delayed because of COVID. But the molds are complete. And we should be getting our first shipment by mid August, they're called spin pods, and basically what they are is little rotating wall vessels that remove all of the hardware, so they're disposable.

About three centimeters in diameter, they hold about three millimeters of culture volume. They're suitable for yeast and mammalian cells, we grow on little carriers. So if they're adherent cells, or in suspension, we just grow them in the pods, and they have breathable membranes. But if you have a typical laboratory roller, like if you're doing an immunoblot, you throw a tube on the roller, you can put these spin pods on a roller, and you get the same kind of dynamics that you would in a rotating wall vessel, but your experiments are $3 instead of 30$ or 50$. You toss them as you complete the experiments. I would hope to be able to share some of that with you, early fall at the latest.

Getting back to your question. I think there is a use for microgravity experiments on Earth, at one G. In order to take the next step, we need more standardization. I don't know what the best simulator is, but it would be nice to have data to make that call.

iGEM Concordia, Lancia 27:21
We would be absolutely interested in checking out your rotating wall vessels, they sound incredible. We are planning on building a clinostat. We had chosen the 3D clinostat to go forward with for our experiments.

Dr. Nislow 27:43
Where'd you get the designs for your clinostat? Or is it something you designed yourself?

iGEM Concordia, Lancia 27:49
We're beginning right now. It was Dr. Richard Baker of CoSE, he had built one and there's also Dr.Edval Rodriguez de Viveiros in Brazil, we're speaking with him on Monday. There are a few people that have built them. We’re going to be communicating with them and then working on the design. We have some team members that are computer engineers or hardware people.

Dr. Nislow 28:12
It might be worth you for your team to approach this shell company, it's a nonprofit called Bio-Serve out of Boulder, Colorado, and we've worked with them quite a bit.

They've built some very simple Clinostats for yeast and bacterial microbes, so they might have some advice. It is basically a very fancy, very well calibrated roller that you put in your incubator. And they tend to be very cheap. They tend to find creative and simple solutions.

iGEM Concordia, Lancia 29:13
As part of our project, because we realized there is this lack of standardization. The solution we had come to was to produce a control strain of yeast. That would predictably, give off a signal in microgravity, so there would be a benchmark that could be used in the industry. What do you think of this idea?

Dr. Nislow 29:45
I think it's great. And I would marry that to once you design the strain, with the reporter, deposit in AddGene or some source where you lower the friction for people using your strain, as opposed to the strain they're using, or at least incorporate your strain into their experiments. In the yeast world going back to the sequencing project, if we hadn't decided on a strain, and the strain that we use is a compromise. I mean, it's not wonderful, but it's the same strain that everyone's using. So that would go a long way. Just as much as the hardware, having a universal strain that people use, at least in addition to their favorite strain.

iGEM Concordia, Lancia 31:16
Beautiful.We’re going to jump a little bit more into the genetics aspect. We want to build a reporter in yeast. We've chosen genes based on their predictability so that when we look at other papers, the results are consistent. We want to perform evolutionary experiments. We're looking at directed, potentially adaptive, we're not sure yet. Then we're hoping to obtain strains that have more tolerance to microgravity-induced stresses in the end, that's our goal. We have chosen some promoters, it took us quite a while to get there. I'm gonna drop them in the chat and out of broad curiosity, do you have anything to say about our selection?

Dr. Nislow 32:08
Yeah, these are all... I should know a lot more about the GAL promoters but I don't. All the others seem very reasonable. If I remember correctly, they're reasonably highly expressed.

There might be one or two I would suggest adding the SOD1 and SOD2,one is cytoplasmic and one is mitochondrial. And I'd be curious why you chose the two GAL reporters.

iGEM Concordia 33:00
As part of iGEM, there's all these criteria we have to meet. One is collaboration. The Toulouse team is also working in yeast to produce vitamin A and yeast, but then they're doing some photosynthetic process to make it taste like rose or mint. We're looking at vitamin A as a proof of concept for a project. So to work with it through collaboration. UCalgary is also working with Vitamin A in yeast. So we wanted to look at those promoters and include them in hopes that if we're successful, we would have microgravity strains that are more tolerant to microgravity-induced stress for vitamin A production.

Dr. Nislow 33:39
Yeah, and it's nice to have. I know these are some of the others are inducible or partially inducible. But the GAL certainly are inducible and repressible. That's nice to have in your toolkit. I think this is a great start. Is the idea to empirically find out which is best and winnow down to a couple? Or two to push all of these forward?

iGEM Concordia, Lancia 34:09
We're looking to do six or eight. This is going into our next question. We're studying how to build a reporter. Assuming this report is successful for building resistant strains, are there any approaches that you would suggest?

Dr. Nislow 34:36
A couple of broad categories to consider is you'd want the brightest one possible. I just don't think there's a lot to be gained from dim reporters. There are some super GFPs that have been optimized for yeast. That's probably the biggest consideration, the next one will depend on your experimental design. The half-life is an important consideration. Do you want a long lived GFP or do you want one that will be degraded and not passed on to daughter cells?

My gut would be a bright and short lived reporter. But, it really depends on the experiment, right? If you're doing a longevity experiment, you'd want the opposite. Wavelengths obviously, there's a trade off between brightness and wavelength. The further red you go, the dimmer you are but the less noise you have. What degree of multiplexing might you want, what might you need? It's something to consider. And then because a lot of space experiments have to be done where you can't analyze until, say, splashdown two weeks later. I think if at all possible, having a fixable fluorescent reporter would be very important.

For example, you perform your experiment on the station or somewhere in outer space. You terminate the experiment by the addition of fixative. But you still leave yourself

the opportunity to sort the cells or enumerate who's bright, who's not bright. I think sorting is going to become a very big part of these kinds of experiments. It's a feeling, not a fact.

I think that when you're dealing with populations of millions, you want to have an ability to identify those rare events that are doing what you want to do. Or if you have a tube with several million cells and you want to find the 1% that are the brightest under that condition. Go and sort them and it's very unlikely that we're going to be able to make them alive again, but at least we can sequence them and find out if there's anything in their genome that tells us why they were in that class.

There's nothing magic about the rules for designing an appropriate reporter. I guess I would say, the logic gate feedback systems, they are a lot more complicated. You might consider keeping it simple to start, but building in the ability to reuse the pieces. Take the classic synthetic layout modular synthetic biology approach, where the thing you build first might not be what you want, ultimately, but at least you can reuse those parts.

NASA loves the KISS principle. Keep It Simple, Stupid.

iGEM Concordia, Lancia 38:33
You had mentioned a bright reporter, what is your criteria for a bright reporter? We've been looking at median abundance in the cell.

Dr. Nislow 38:41
Yep, that's just all there. That's what we'd look at. So either by imaging or by cytometry.

iGEM Concordia, Lancia 38:49
If you're looking at median abundance in the cell, what do you use as your threshold for the reporter?

Dr. Nislow 38:57
We're very empirical. Can we reliably identify the top? In a flow experiment, can we reliably gate the top 5% of the population? I guess, does that reporter have enough dynamic range to slice up the population into many bins?

iGEM Concordia, Lancia 39:31
With respect to an evolutionary process, do you have any gut feelings? What would be more successful for us? We're looking at adaptive evolution as one option, and then we're looking at directed evolution. If you have any other advice for approaches we could take into consideration.(Note: time signatures shift)

Dr. Nislow 7:21
Well, for adaptive evolution to what extent do you rely on robotics?

Can you automate it to reduce the number of times a trainee has to go in and has to interact with the experiment,? Six months is enough for many hundred generations. I feel like 2-300 generations is a reasonable amount of time, if you're going to see a change. In terms of directed evolution, we haven't done very much. We're doing something that's a little bit adjacent to that, in that we're taking yeast drug targets that are homologous in yeast to human drug targets. We're trying to randomize the sequence as much as possible with these variant libraries that you can purchase and then screen for variants that have either sensitivity or resistance to a particular drug. If I had to choose, I think adaptive evolution is much more in the spirit of Omics in that you're letting the cell tell you what it needs to survive in that condition. And there's definitely a place for the directed evolution, but it would be more when you have good candidates.Is the focus strictly on microgravity or are you looking at radiation and microgravity at the same time? Is your focus really microgravity?

iGEM Concordia, Lancia 9:36
We are focusing on microgravity. I did want to ask you that question. We have spoken with Ice Cube Services, some of the commercial companies that take care of sending your experiment to space. Radiation is something we know that other people have dealt with. We wanted to ask how do you address radiation when sending an experiment to space?

Dr. Nislow 10:00
We don't anymore because, frankly ,the radiation that you experience a lower Earth orbit is pretty modest. I think you're safe. We're focusing 90% of our radiation experiments on good ground control experiments. Going to Japan and Germany and doing as many ground experiments as possible. I think the focus on microgravity makes a lot of sense.

iGEM Concordia, Brian 10:45
I want to jump in and expand. Early on, when we were just starting off the research, I looked a bit into directed evolution that also talked about radiation. What they were saying and that might be interesting, is to duplicate certain genes that you are interested in, and then let one gene carry out the traditional function, you will have less risk of the function of that gene not being affected by radiation. And then because you have to have the genes, one gene would be almost freed up to try evolutionary approaches to solving the issue while maintaining the function of the original gene. Have you heard anything about that?

Dr. Nislow 11:30
Well, not directly, but I think it's an excellent idea. We always work with diploids, partly for that reason, because if you're looking to evolve a new function and that gene is essential for viability. You're asking an awful lot to evolve the new function, but wait, don't lose the functions that are required to keep the cell alive. If you have two alleles, or in what you just mentioned, if you duplicate intentionally, you give room for what they call neo-functionalization over time. Either work with diploids or work with strains that you've duplicated the genes of interest.

iGEM Concordia, Lancia 12:25
There are ground controls, you send your sample to space, and then I had read about having 1g controls in space. Can you speak to that at all? Is that something that's familiar?

Dr. Nislow 12:41
We have never done 1g controls in space. What we've done is ground and they're usually slightly asynchronous in that we wait for the crew to start and then we start. What we do have are flight grown and flight not grown. It doesn't address… in that matrix there's one missing window and that is 1g controls in space. I'm actually sure there is a centrifuge on the ISS to recreate 1g. It's just access. Everything on the station, you have access issues, but more importantly you have crew time issues, they have no time. Everything they do from going to the bathroom to starting your experiment is scheduled. The more autonomous you can make your experiment the better.

iGEM Concordia, Lancia 14:05
In your yeast genome deletion paper, there were experiments with NaCl? If you could elaborate on that and why you chose to do so?

Dr. Nislow 14:25
The rationale there, jumping back 10 years is that we expected some kind of a cell wall, a cell membrane response. It's known from our work and others that sodium chloride induces a very strong cell wall integrity response. We wanted to see if the magnitudes of that response were similar to the magnitude from microgravity and this could be a 'be careful what you offer situation for', we have about 20 times the data that we haven't published as that we have. We have sodium chloride, we have all kinds of different drugs. This could be depending on your bandwidth, we could share many of those experiments that you could mine de novo and see. I think it might be nice to get the computational folks on your team up to speed just looking at that data, and again, it goes back to 2011. It's a function of we don't have the bandwidth or the funding to look at all those. To get a study to publication takes several people. That's something we could discuss or figure out how to transfer to you guys to post.

iGEM Concordia, Lancia 16:15
That would be incredible.

Dr. Corey Nislow 16:18
You know with genomics, there's the paper, but then there's always the data that everyone uses to write the other papers and we tend to ignore that. I'm a wet bench person, so I tend to ignore it. That's a bad excuse.

iGEM Concordia, Lancia 16:36
At the beginning, you had mentioned synergizing with our project. I know you have a couple of things like you had spoken about the rotating wall vessels, the data, we would absolutely be interested in doing something with that. I'm not sure if you had anything else in mind, or if you have questions for us.

Dr. Nislow 16:54
We should have another meeting where you guys show me your project. At whatever level of granularity you'd like. What's your plan for sequencing, what tools do you have available? I know Genome Quebec is a great institution. I would hope you'd have access to all the latest greatest sequencing technology but some of the sample prep things we would be happy to advise on. And there is one issue that people don't like to talk about. That is RNA later is the bronze standard for how you stop cells. You can harvest the RNA later when things return to Earth, and it's not a great preservative. A better preservative, surprisingly, is just formaldehyde. You fix it. Then when it comes time to isolating the RNA, you reverse the cross links and go, but there are issues in that NASA is not happy about sending formaldehyde out. That's actually a very simple, but very important benchmarking experiment to do is; what are the effects of RNA later? And what other protocols might we think about?

You guys probably know space biology has a mixed reputation and part of it has always been that you can't go and reproduce your experiment. But that's no longer true. We've sent the same payload multiple times. Now the bar goes higher and if you're going to make a drug in a synthetic organ in a synthetic pathway that you put into yeast in microgravity, what level of quality control do you have? There's a lot of logistics and processes that need to be touched on.

[End Part 1]

Dr. Corey Nislow

Part 2: August 7th with Dr. Aashiq Kachroo & iGEM Concordia

Dr. Nislow 0:00
We know that dehydration is not a perfect way to carry yeast for long term storage. It does increase your flexibility if you can take your engineered strains and freeze dry them. And I just was curious if that's something that you're considering.Say you have a product way down the road and Artemis 4 is launching and going to Mars. If the company comes back and says, we need your payload to be freeze dried. I just was wondering if you had that in your design, because we've been forced by NASA to take the deletion collections and show that you can take the whole collection freeze dry it, store it under desiccating conditions and hydrate it after launch, and show that the performance of the collections is similar. If you need to go that route, they're very simple protocols. You add a long chain carbohydrate and you slowly freeze dry. It was a pleasant surprise for us.

Dr. Kachroo 2:01
Mixture of lyophilization and phrasings.

Dr. Nislow 2:04
Exactly. And it's probably no surprise, because when you go to the store, you buy your yeast that way too, right? Yeah.

Dr. Kachroo 2:13
Yeah. But the scientific question that we can ask is can our yeast be freeze dried and stay viable after you take it out from there.

Dr. Nislow 2:21
I think it would be nice to be able to show that, to what extent, how viable are they and it just gives you more flexibility when you're thinking about design constraints for SpaceX and everything. Yeah, that's a really important question.

iGEM Concordia 2:39
We're looking at strains right now. We are a little bit curious if maybe there aren't already strains that are more optimal for what we're doing. And are you familiar with any strains that already exist that are better suited for microgravity?

Dr. Nislow 3:01
Not really we've been using the BY modification of S288C... Aashiq, do you know of a better, more generally, tolerance strain.

Dr. Kachroo 3:19
Not that I know but everyone is using classical S288C or BY 4741. Most of the collections that we have in the labs are in endogenetic backgrounds. I have been telling them that we already have several of these collections. And for their use, they might use a GFP version of the selection, which has a reporter already inserted at every gene. So you can actually pick anything you want and study that. I think Chris Brett's lab has that collection. So you should be able to get your gene of choice on that collection and study that.

Dr. Nislow 3:55
If you needed a justification for the lab strain over other strains, whenever we go and test a wild Saccharomyces, even though there's a debate whether there are any true wild strains as opposed to escape strains. But if you go and look at a non-laboratory domesticated strain, almost across the board, they're more sensitive to different stresses. So you're probably already ahead of the game by using the lab evolved strain.

Dr. Kachroo 4:31
In future if you want to really extend your basic work to really application you can think about people might want to take fermenting yeast or beer-compatible yeast, you know, people who make beer, they use a special type of use, they don't use the yeast that we work with in the lab. So those are a mixture of really evolved strains, in principle. They are adapted to making really good flavors in your beer and you would want to study them in your context of space quest. So essentially bring the use of beer, a mixture of the strains or whatever that is, and study their effect on microgravity. Would that make it an ideal beer and both ferment bread as normally as in microgravity conditions, that would be more application based.

Dr. Nislow 5:22
Building up flexibility and I think your point, another way of saying your point is that there won't be one strain to solve all the problems. You know, there'll be one beer strain, one bread strain, maybe one strain for making pharmaceuticals.

Dr. Kachroo
So you might have to really standardize for every process that you want to study in space, you'll have to use a special use for that. So that'll be great, because people might maybe take yeast for making beer in space, and they would one day go to Mars and make beer. We're scientists. They might have to make bread. This drives what we get from the market.

Question is, are they stable in those environments? Can we use them? And can we modulate and modify them? So basically classical genetics, what you do if you study a promoter, or suggest genes that might be changed to avoid the stress, and then you apply that on to the yeast, which we actually use for making food or bread or beer, say whether that will apply to them as well, because they are their yeast but they have different mutations. They have different genetic regulations, but important, test them on those strains as well and apply them on the strains.

[Dr. Nislow drops off the call]

iGEM Concordia 7:06
What I understand, the lab strains are a lot of reasons to use them, as scientists we are pretty familiar with them. But while we're researching to make sure that it's well defended, if we were looking at other strains, we should be looking at our application. If we're looking at vitamin A, we should look at strains that are used for that.

Dr. Kachroo 7:24
So the question is, look, they all are similar. They're yeast, but they have accumulated mutation. In fact, beer yeast that you talk about, they're not a single use strain. It's a population of different types of nutrients in the population that the yeast. The question is, let's say you have a gene x, if you knock out that your microgravity effect is nullified, we don't really see that effect. Will that same mutation also result in similar behavior in a totally different genetic background with all sorts of things, different mutations, will it give the same result? So that'll be really good to test it. Wild type strains in the lab to tell you likely genes, you have to change your modulation to get a certain effect. Then you can apply that information to the yeast that we actually use in food, industry, fermentation, pure... then ask this question because you're really talking about two different genetic backgrounds at that point.

[Dr. Nislow rejoins the call]

Dr. Kachroo 8:56
So if you really want to go after yeast strains for beer and all, I think I have a colleague in Austin, we can actually get a sample of that. But that will be probably at the end of the project where we actually apply our work on the yeast that we might want to take with us.

Dr. Nislow 9:24
Is there a flight component to this project?

iGEM Concordia 9:31
Not yet. we have a long way to go. We have spoken with people about collaborating for funding, so we're aware of the options. We spoke with Hilde Stenuit from Ice Cubes Service, they will help us find grant funding and collaborations. They work in collaboration with the European Space Agency. Yeah, and also CSA (Canadian Space Agency) but it's 40,000 dollars to get it up. Even if we collaborate, maybe we're lucky we could get it down to 10. We definitely have to do the work first. It's on the back burner.Did you have any other questions after our presentation?

Dr. Kachroo 10:24
I actually had a question quite early. Can you give us an idea of what genes to look at, to begin with? When I was telling them to pick 10-20 genes and study them. 20 candidate genes that would allow us to check.

Dr. Nislow 10:41
The two pathways that come to mind, and there are a number of genes, of course, in each of these pathways, but the Osmoregularity pathway, the HOG pathway, it comes up in many of our experiments. This is a both a regulatory pathway and a stress responsive pathway. I think it'd be useful there. The genes involved in managing oxidative stress in yeast. So, SOD1, SOD2, Glutathione... and the reason is, well, oxidative stress, we don't know. The reason it's relevant in microgravity, some people have speculated that it has to do with the fact that in the absence of gravitational flow and fluid flow within and outside of cells, the cell does not get rid of the products of oxidative stress and will have a negative reaction to that. I would also consider the HOG osmolarity pathway, the oxidative stress pathway, the cell wall integrity pathway, in particular BCK and SLT2 would be genes I would look at.

And then looking farther ahead, internal stresses, if you're going to be building a yeast cell factory, it may not be directly relevant to microgravity, but you'll want to consider a reporter for proteotoxic stress. At what point you've pushed the cell to just make too much of a particular protein, Aashiq, I'm sure you've seen that when you're asking us yeast to make a human protein. You probably get that response at times.

Dr. Kachroo 12:53
Correct, these are really important and because there are
6000 genes in yeast you have to pinpoint and start somewhere.

Dr. Nislow 13:01
Those are my four favorites and then I would always suggest including a histone control. So a reporter that will not vary and either a histone or a ribosomal control.

You don't want one where the levels are so high that the signal from that reporter is going to blow out your test reporter, but you do need something, it would be nice to have the ability to normalize.

Dr. Kachroo 13:30
Excellent. Okay, so I have been telling them to really classify their pathway on classical gene expression profiles. And then basically take that data and put them in the pathway specific effects, is there any pattern which is upregulated, not just one gene but the entire system is upregulated? So yeah, look at it as a pathway rather than a single gene.

Dr. Nislow 13:57
I look at this as a classic genomics problem. You have 6000 genes. You have got to use the information available to get down to a manageable number. Then for the purposes of getting funding and getting people interested, you need to do everything you can for that small set of genes to justify why them and not the others. I'm sure you've gotten your grant proposals. Well, you're looking at these 200 genes, why not those 200 so once you winnow it down, you need to justify it.

Dr. Kachroo
Yes. So we do see proteotoxic stress and whenever you express something really highly abundant. Yeast is a hackable system. We have found yeast as accommodating in terms of entering stress, but for the yeast strain, which you want to do something with which you want to read and being wrong... You want to make sure that they're not under stress which can cause problems. So yeah, those are really excellent points here. But regarding the proteotoxic reporter, we are making one for proteosome. But that's a separate project that has nothing to do with iGEM.

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Dr. Nislow 16:12
I had one other thought while you guys were talking about funding. It may be premature, but there's a university adjacent group called Creative Destruction Labs. They were founded in Toronto, and then quickly, the second site was Vancouver, and now they're in about six or eight different cities. And the reason I bring it up is that Commander Hadfield was leading. Basically it's an accelerator mentorship program where they

take in very early stage companies. I'm not saying you're a company yet, but you're looking at products and even if they say well, it's too early, it might be worth approaching them. Because they are very keen on, Canadian born ideas in companies and it's also, just as a fanboy, it's a great opportunity to talk to Commander Hadfield. There's a space stream. You'll definitely get something out of it.

And a lot of the companies that apply to this space stream are looking at cheaper, faster ways to get payloads into orbit. So they're on the launch side of things. It's always helpful when you're developing a launch capability to show that a simple experiment can be taken aboard and that's one of the attractions of yeast experiments is that, although they're very sophisticated in design, in logistics, they're fairly straightforward. Anyhow, it's just kind of a high level idea that maybe looking at CDL space stream. I'm sure it's just a group of scientists and entrepreneurs that want to help early stage companies.

Dr. Kachroo 19:10
Excellent. Talking of the simplified experiment, always really think about how to make something very simple. What is the simplest data you can get from your experiment? I know we can all make highly complicated or even maybe smart, better strategies, but because this is a space specific rule; they have minimal design, they have minimal instruments. So there you probably have one small tube to give to them. So think about making your proposals to iGEM simple. This is a simple assay for a space specific role.

Dr. Nislow 19:49
For Example, we initially designed a yeast growth chamber where a crew member would inoculate and then terminate the experiment by the addition of fixative, or RNA later. Now what we do and what we're doing with Artemis is we have the yeast collection in a pill. And it's crushed automatically after two days, and the experiment terminates because yeast uses up the carbon source. So basically, you initiate the experiment by crushing the pill and then it self terminates. So as we get fancier and fancier on the missions, the experiment gets simpler.

Dr. Kachroo 20:39
Talking about this pill. You said collection, I guess you're sending all the knockout collection there and seeing which wins?

Dr. Nislow 20:49
Yeah, we're sending the knockout and the overexpression collection.

Dr. Kachroo 20:52
Do you see behaviors which are favorable?

Dr.Nislow 21:00
We haven't analyzed much of the flight data yet. Right now what we've been tasked with is not so much microgravity, we are tasked with looking at cosmic radiation, because that's the next thing.

Because once you leave Lower Earth Orbit (LEO), then the cosmic radiation becomes substantial here. So that's what we're really focusing on those reporters. That's why I thought it was a nice synergy between what you guys are doing and what we're doing, because when you actually get there, the cosmic radiation experiments are mostly done in Germany and Japan in their particle accelerators. So 95% of our work is ground controls. I think that by the time Artemis launches, which is supposed to be April of 2021, we both might have gotten quite a bit farther along.

Dr. Kachroo 22:08
Excellent. The team should really consider what expression collection do we have access to? We should try to use that, because they might actually survive better in certain kinds of genes that are abundantly expressed.

Dr. Nislow 22:22
Replication is really powerful. Just because we've done that once or twice or three times, having you guys follow a similar protocol and doing exactly the same thing will make it so much more powerful. I'd be happy to, and I will share our freeze drying protocol. I'll send you the presentation we did at the Gravitational Society meeting. which just shows all the hardware ASGSR, the American Society for Gravitational and Space Research.

[End part 2]

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